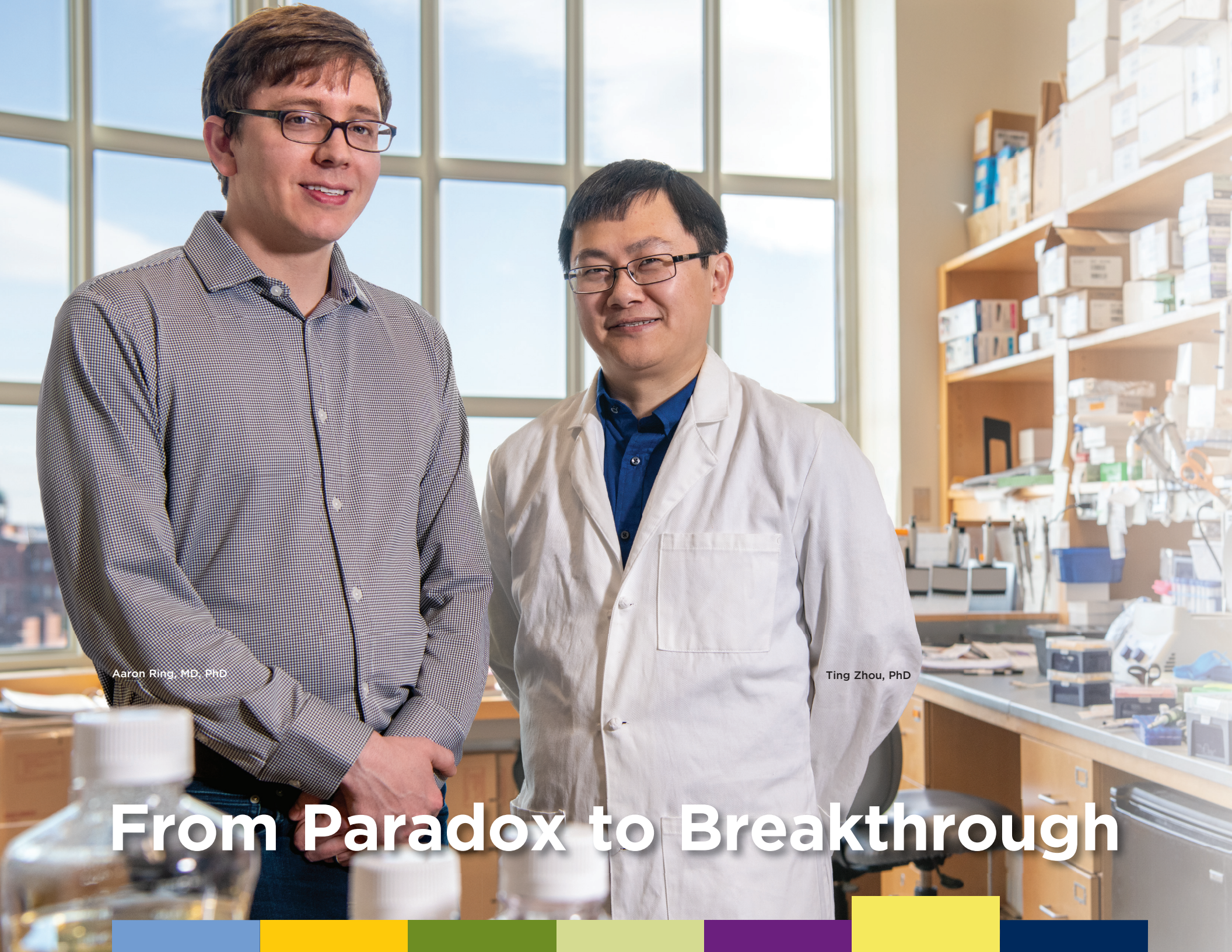

From Paradox to Breakthrough

Aaron Ring, MD, PhD, Assistant Professor of Immunobiology, was hooked by a paradox. He had been studying cytokines, hormone-like proteins that control immune responses, to understand their potential to stimulate anti-tumor immunity. Though cytokines such as interleukin-2 (IL-2) have been in clinical use for decades, they have historically shown only limited effectiveness.

To look for potential new cytokine therapies, Dr. Ring took a bird's-eye view to see if any cytokine pathways had been overlooked. He had been intrigued by a study that used cutting-edge "single-cell" profiling to identify the genes most closely associated with tumor-infiltrating lymphocytes (TILs). His lab used the study's findings to analyze every cytokine pathway that could be detected in the data. Dr. Ring was hunting for interleukins that could deliver a potent, but specific signal to activate TILs.

"Looking at it from the standpoint of someone trying to hack into the immune system and turn on a response," he said, "we found that the IL-18 pathway had the desirable features. It appeared to be an 'open port' on these elite anti-tumor T cells."

Since IL-18 seemed so promising, Dr. Ring and Ting Zhou, PhD, a postdoctoral associate in Dr. Ring's lab, dove into the clinical data surrounding it. They found a set of clinical trials in which cancer patients had received high doses of the cytokine. "It shocked us to find that IL-18 failed— there were no responses in several dozen patients tested," said Dr. Ring, "It was an incredible paradox. How could this powerful cytokine pathway be so ineffective?"

They learned that IL-18 had a 'decoy receptor,' IL-18BP, which was produced at very high levels in tumors. Dr. Ring and Dr. Zhou hypothesized that IL-18BP counteracted IL-18 and curtailed its power to elicit immune responses—sensible in healthy people but self-defeating for fighting cancer. They thus set out to build a molecule that could evade IL-18BP and thereby unleash full IL-18 activity in the tumor microenvironment.

To approach this difficult problem, they used a method called 'directed evolution.' "Sometimes we can't settle for nature's solution," explained Dr. Ring. "We have to create our own. The odds were stacked against us. IL-18 has evolved to be tightly regulated by its binding protein and IL-18BP plays an important role to protect us from runaway IL-18 activity and autoimmune disease."

They created a large collection of genetically-modified yeast in which each yeast cell presented one unique variant of IL-18 on its surface. Using magnetic and fluorescent cell sorting, they screened about 250 million variants, looking for those that retained the binding for IL-18's receptor but didn't bind to the decoy, IL-18BP. They repeated the process for several weeks until they pinpointed the best candidate. This became their synthetic 'decoy resistant' molecule, DR-18.

They then tested DR-18 in mouse models, including melanoma tumors in collaboration with the laboratory of Marcus Bosenberg, MD, PhD, Professor of Dermatology, Pathology, and Immunobiology. What came next, said Dr. Ring, "was a eureka moment. The activity of DR-18 in these tumor models in mice was much stronger than anything I'd ever seen. It was like flipping a switch." By contrast, the tumorous mice that received normal, or 'wild-type' IL-18 showed no response, just like patients in IL-18 clinical trials.

"IL-18BP sends a jamming signal that prevents the activation of lymphocytes in the tumor," he said. "With DR-18 we made a version of IL-18 that can't be jammed."

In many mice, the tumors entirely disappeared. But Dr. Ring was even more excited by the underlying immunological effects. As expected, T cells jumped into action, but the innate immune cells also showed major changes, including activation of natural killer (NK) cells.

Dr. Ring notes that anti-PD-1 immunotherapies succeed partly by activating these stem cell-like T cells, though without increasing their number. By contrast, DR-18 boosted the number of these cells more than fivefold. When Dr. Ring reintroduced tumor cells into mice whose cancer had disappeared, tumors didn't return. The mice evidently were protected by their augmented memory cells.

It's often said that anti-PD-1 immunotherapies take the "brakes" off of the immune system. "DR-18," said Dr. Ring, "steps on the gas. It doesn't remove a negative signal, it provides an activating signal."

Equally exciting, Dr. Ring found that DR-18 worked against a subset of tumors that have become resistant to anti-PD-1 therapies. DR-18's mechanism seems to stimulate the immune system in ways that other therapies don't. He has started a company called Simcha Therapeutics to attract investment that is needed to advance the new molecule into human trials in early 2021.

"Moving beyond discovery is the biggest challenge but also the most rewarding aspect of our work," he said. "And Yale is an awesome place to do this kind of translational research."